

# Denitrification potential and organic matter as affected by vegetation community, wetland age, and plant introduction in created wetlands

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## Abstract

Denitrification potential and organic matter in soils were compared in three different vegetation communities—emergent macrophyte, open water, and forested edge—in two ten-year old created riverine wetlands. Organic matter (OM), cold water extractable organic matter (CWEOM), anaerobic mineralizable carbon (AnMC) and denitrification potential (DP) varied significantly ( $P < 0.05$ ) among vegetation communities. The surface (0–9 cm) soils in the emergent macrophyte community showed highest DP ( $0.07 \pm 0.01 \text{ mg N h}^{-1} \text{ Kkg}^{-1}$ ), OM ( $84.90 \pm 5.60 \text{ g kg}^{-1}$ ), CWEOM ( $1.12 \pm 0.20 \text{ g kg}^{-1}$ ) and AnMC ( $1.50 \pm 0.10 \text{ mg C h}^{-1} \text{ Kkg}^{-1}$ ). In the deeper layer (9–18 cm), DP and CWEOM ( $0.04 \pm 0.01 \text{ mg N h}^{-1} \text{ Kkg}^{-1}$  and  $1.13 \pm 0.20 \text{ g kg}^{-1}$ ) were significantly higher in the open water community than in the emergent macrophyte, and the forested edge communities. Plant introduction did not affect denitrification potential or organic matter content and characteristics. After ten years of wetland development, mean DP increased 25 fold in the surface layer (from 0.002 to  $0.053 \text{ mg N h}^{-1} \text{ Kkg}^{-1}$ ) and 15 fold in the deeper layer (from 0.001 to  $0.015 \text{ mg N h}^{-1} \text{ Kkg}^{-1}$ ). Organic matter content more than doubled 10 years after the wetlands were created to  $90.80 \pm 19.22 \text{ g kg}^{-1}$  in the upper layer and increased 38% in the lower layer to  $46.93 \pm 3.85 \text{ g kg}^{-1}$ . In the surface layer, CWEOM and HWEOM increased 2.5 and 2.7 times respectively from 1993 (pre-wetland conditions) to 2004; in the 9–18 cm layer they increased 1.25 and 3 times, respectively. AnMC increased 4 times in the 0–9 cm layer but it did not increase in the 9–18 cm layer. Humic acids were the most abundant form of organic matter in 2004 and 1993 samples. Significant ( $P < 0.05$ ) positive relationships between DP and OM, CWEOM and AnMC were found in the surface layer; in the 9–18 cm layer, significant positive relationships were found between DP and CWEOM and AnMC.

## Introduction

Created wetlands are effective, simple and economical systems for the reduction of nitrate contamination from agricultural runoff (Horne 2000; Mitsch et al., 2001, 2005b). Nitrate removal from the water column in wetlands is carried out by plant uptake and microbial transformation that include immobilization and denitrification. Plant uptake and microbiological immobilization result in temporary storages in the system since most nitrogen will eventually

return to the wetland via plant death and decomposition. In contrast, denitrification constitutes a real nitrogen sink because in this process bacteria reduce  $\text{NO}_3^-$  to nitrogenous gases ( $\text{N}_2$ , NO,  $\text{N}_2\text{O}$ ) that are emitted to the atmosphere (Clement, and Marmonier, 2002).

Heterotrophic denitrification is carried out by soil facultative anaerobe bacteria, which under anoxic conditions are able to perform activity by using nitrates as a final electron acceptor. Organic carbon is required as an essential electron donor for heterotrophic denitrification. Organic carbon availability is one of the most important factors that affect denitrification activity in soils (Beauchamp, et al., 1989). Quantity and characteristic of organic matter in soils is influenced by the amount and type of vegetation (Boyer and Groffman, 1996). Several studies have investigated organic matter availability in agricultural and forest soils (Burford and Bremner, 1975, Bijay-Singh, et al., 1988, Boyer and Groffman, 1996), and riparian buffer zones (Hill, and Cradaci, 2004, Rotkin-Ellman et al., 2004). In created wetlands denitrification rates have been studied (Poe et al., 2003; Srivedhin and Gray, 2005; Teiter and Mander, 2005), however, little research has been done to investigate the influence of characteristics and availability of organic matter on denitrification activity in created and restored wetlands.

Creation and restoration of wetlands for nitrogen removal from agricultural landscapes in the Mississippi River basin has been proposed as a mean to mitigate the hypoxia problem in the Gulf of Mexico (Mitsch et al., 2001, 2005b; Mitsch and Day, 2006). In these created wetlands, high nitrogen removal rates are desirable. Therefore, understanding factors that affect denitrification rates in created wetlands receiving non point source pollution is important to optimize their ecological design and performance.

In studies carried out in microcosms and constructed wetlands treating wastewater, different nitrogen removal efficiencies have been observed when wetlands are planted with different species of macrophytes (Lin et al., 2002; Bachand and Horne, 2000; Hume et al., 2002). These studies have suggested that the differences might be due to the type and quantities of organic carbon that the plant species provide.

In this study we investigate if links exist among vegetation communities, quantity and characteristics of organic matter, and denitrification potential in the substrate of created marshes in Midwestern USA. Our objectives were to

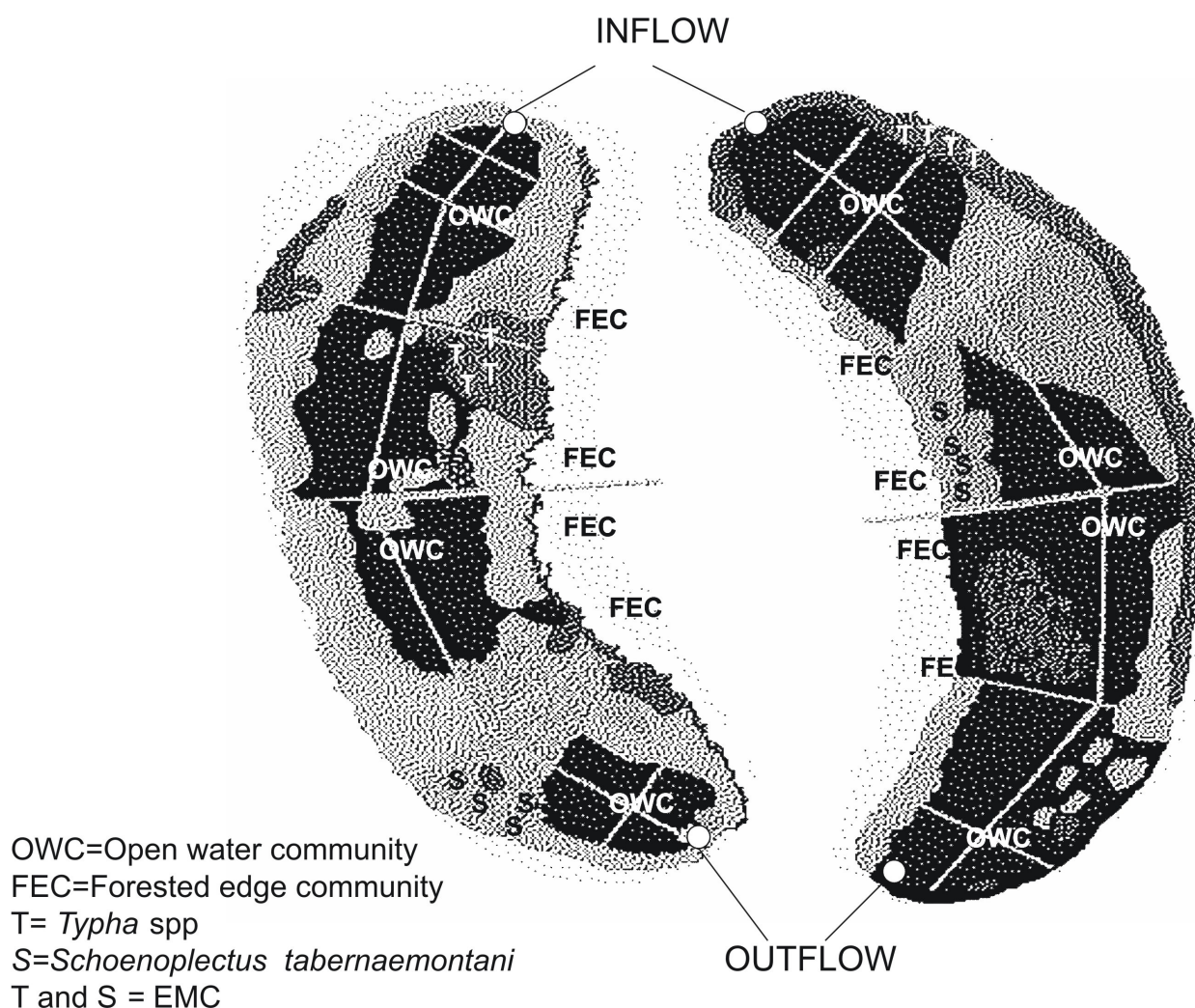


Figure 1. Two 1-ha experimental wetlands at the Schiermeier Olentangy River Wetland Research Park (ORWRP), at The Ohio State University, Columbus, Ohio. Letters represent soil sampling plots in the different vegetation communities.

compare organic matter content, fractions of organic matter, and denitrification potential in plots covered with different vegetation communities in two created marshes (one planted and one naturally colonized). Because soil samples were archived when the wetlands were created, we were also able to compare these soil parameters to those of non-wetland soil samples taken before the wetlands were created.

## Materials and Methods

### Site description

The study was carried out at Olentangy River Wetland Research Park (ORWRP), which is located in the north campus area of The Ohio State University in Columbus, Ohio USA. Two 1-ha experimental wetlands were constructed in 1993 on alluvial old-field soils adjacent to the third order Olentangy River. The primary original soil type at the experimental wetlands is a Ross (Rs) series soil, which is a floodplain alluvial soil that ranges from silt loam to silty clay loam to loam (Mitsch and Wu, 1993). Both wetlands have deeper water sections located in the north, central

and southern positions of the basins. The open water area of the wetlands is subdivided into 3 deep (approximately 60 cm) basins, inflow, middle and outflow, all surrounded by emergent plants. Water has been pumped through these wetlands since March 1994 and both wetlands have received the same amount of water and had the same flow patterns since they were created. In a whole-ecosystem experiment one wetland (W1) was planted with 12 plant species in May 1994 while the second wetland (W2) was naturally colonized by macrophytes. See Mitsch et al., 1998, 2005a, c for a description of the planting experiment and the development of plant communities in these wetlands.

### Soil sampling design

Four soil cores (10 cm diameter x 18 cm depth) were taken in two patches of the emergent macrophyte community (EMC), in the open water community (OWC) and in the forest edge community (FEC) of each wetland (Figure 1). Single species patches of emergent vegetation were *Typha* spp. and *Schoenoplectus tabernaemontani*. The open water zone was colonized by floating and submerged macrophytes such

as *Lemna spp.*, *Potamogeton pectinatus* and *Ceratophyllum demersum*, and by algal metaphyton. The edge zones were colonized mainly by woody species such as *Populus deltoides*, *Acer rubrum*, *Salix nigra*, and *Acer negundo*. In the emergent zone, the criterion for choosing a patch was that the plant species was dominant in the patch and was present there for at least two consecutive years. In order to establish sampling plots, maps of vegetation communities in these wetlands were utilized to verify what species were present (Mitsch, et al., 2004). In the emergent vegetation patches, four soil samples were taken in random locations in an area of approximately 2 m<sup>2</sup>. In the open water and edge zones, soil samples were taken near the inflow (one sample), in the center (2 samples), and near the outflow (1 sample). A total of 16 soil samples in each wetland were taken in late autumn 2004. A change in consistency of the soil occurred in the samples at approximately 9 cm depth; thus cores were separated in two subsections—0–9 cm and 9–18 cm depths. Soil cores were placed in zip plastic bags and refrigerated at 4°C. Each core was divided into 4 parts. One quarter was weighed and dried at 105°C to constant weight to determine bulk density. The other three quarters were mixed together and homogenized by hand; roots, dead plant material and twigs were separated. In this homogenized sample, soil moisture, chemical analysis and incubations were performed. Soil moisture, denitrification potential (DP), total organic carbon and fractions of organic carbon were analyzed in triplicate for each sample.

### Denitrification potential and mineralized carbon

Denitrification potential rates were measured within 45 days after sampling, using the acetylene block technique, which inhibits the final conversion of N<sub>2</sub>O to N<sub>2</sub> (Tiedje, 1982). Samples of homogenized fresh soil (approximately 15 g dry weight) were placed in 1000 ml Mason jars; each jar had a gray butyl septum for gas sampling and a 15 cm sealable vent tube (tygon 2 mm i.d.) attached to the lid. Fifty ml of nitrate (10 mg l<sup>-1</sup> of N as KNO<sub>3</sub>) were added to each sample. The jars were closed and flushed with oxygen-free N<sub>2</sub> for 2 minutes at a flow rate of 8 L min<sup>-1</sup>; this was done to provide anoxic conditions. While the jars were flushed with N<sub>2</sub>, the tygon tube was open and submerged in water; when flushing was finished, it was closed with a small clamp and 10% of the volume was replaced by acetone free acetylene. The slurries were incubated at 20 ± 30°C, and headspace gas was sampled at 0, 6, 12, 24 and 30 hours. The jars were shaken by hand approximately every 3 hours, and before the gas sampling. N<sub>2</sub>O was analyzed using a gas chromatograph (Shimadzu GC-14-A) fitted with a 2-ml sampling loop, two Porapak-Q 1.8-m columns and a <sup>63</sup>Ni electron capture detector. The instrument used ultra-pure nitrogen carrier gas (10 ml min<sup>-1</sup>), and operation temperatures were 40, 80 and 300°C for column, injector and detector, respectively. CO<sub>2</sub> was analyzed using a Porapak-Q 1.8-m column and a thermal conductivity detector with ultra pure helium as the carrier gas (25 l min<sup>-1</sup>) at temperatures of 40, 40 and 200°C for column, injector and detector, respectively. Total

denitrification rates were calculated from the linear portion of N<sub>2</sub>O produced over the sampling time, and anaerobic mineralized carbon from the linear production of CO<sub>2</sub> (Hill and Cradaci, 2004). Gas concentrations measured in the headspace (N<sub>2</sub>O and CO<sub>2</sub>) were adjusted for the gas in solution using the Bunsen solubility coefficient (Tiedje, 1982). The nitrate concentration remaining in the slurries was analyzed calorimetrically by the cadmium reduction method using a FIA Lachat AutoAnalyzer. Final concentrations were always > 2.0 mg l<sup>-1</sup> indicating that nitrate was not limiting during the incubations.

### Physical and chemical analysis

Soil moisture was determined by drying field moist soil samples at 105°C until constant weight. Total organic matter was analyzed by loss on ignition at 550°C for 1 hour according to Nairn (1996) and described in Anderson et al. (2005). Open water samples had slight alkaline pH, thus, in order to avoid carbonate interferences, these samples were pretreated with 10 M HCl until no bubbles were observed, then they were dried at 105°C and combusted as described before.

Labile and stable organic matter was determined using the sequential organic matter extractions, according to Nguyen (2000). Organic matter fractions were sequentially extracted by shaking approximately 2 g (on dry weight base) of field moist samples on an end over shaker (120 rpm) with 30 ml of cold (21–23°C) distilled water for 18 hours; then with 30 ml of hot (80°C) distilled water for 18 hours. To quantify stable organic matter, the remaining residue was subsequently extracted once with 30 ml of a mixture 1:1 of 0.1 M HCl and 0.3 M HF for 8 hours, then once with 30 ml of sodium pyrophosphate for 24 hours, and finally twice with 30 ml of 0.5 M sodium hydroxide for 24 hours. Between each step, samples were centrifuged for 15 minutes at 5000 rpm and the supernatant was filtered (45 minutes), separated and saved for total organic content (TOC) analysis. To establish the time for each extraction of stable organic fraction, in a trial set of samples, extractions were performed several times and extracted carbon was analyzed. In the case of acid and pyrophosphate extraction, it was found that a single extraction was sufficient, but for sodium hydroxide extraction it was necessary to perform the extraction twice. Soluble organic carbon in each extract was analyzed in a Shimadzu TOC-50A50A analyzer. Humic acid precipitate was redissolved in 200 ml of water at pH 7 and soluble TOC was analyzed. TOC in the extracts was converted to organic matter multiplying it by Van Bemmelen factor (1.724) to express the results as a percentage of organic matter.

For the analysis before wetland creation, air-dried archived soil samples taken in 1993 by Nairn (1996) and described in Anderson et al. (2005) were utilized. A total of 12 soil samples from 1993 were analyzed. Four 1993 samples, matched in location with 2004 samples taken in the open water zone of W1, the *Typha* patch in W2 and the *Schoenoplectus tabernaemontani* patch in W1, were analyzed for total organic matter, denitrification potential



and fractions of organic carbon. Homogenized 2004 sub-samples were also air dried (2 weeks at room temperature) and reanalyzed (OM, DP, CWEOM, HWEOM and AnMC) to compare with 1993 samples. This was performed to minimize differences for sample treatment. For humic substances extraction, 2004 dried samples of *Typha* spp. patches were analyzed and the results were compared with field-moist extractions. Results of humic substances in air-dried samples were not significantly different from humic substances extracted in field-moist samples; thus, air-dried 1993 samples were compared with field-moist humic substances extractions in 2004 samples.

### Statistical analysis

Statistical analyses were performed with SPSS version 11 for Macintosh and version 12 for Windows. Kolmogorov-Smirnov, Lilliefors and Shapiro-Wilk's tests were used to check normality. The data fit normal distributions. One-way analysis of variance (ANOVA) with Tukey HSD multiple comparison tests were used to detect differences among the vegetation communities, depths and wetland age. When variance was not homogeneous Games-Howell multiple comparison tests were used. Relationships between DP and organic matter fractions were examined with Pearson product moment correlation. A 5% significance level was used to assess differences among treatments.

## Results

### Denitrification potential and organic matter in 10-year-old wetland soils

Denitrification potential (DP) varied significantly ( $P < 0.05$ ) among the vegetation communities and depths (Figure 2). In the 0-9 cm layer, the highest DP was observed in the emergent macrophyte community ( $0.07 \pm 0.01 \text{ mg N h}^{-1} \text{ Kg}^{-1}$ ) while in the 9-18 cm layer, the highest DP was observed in open water community ( $0.04 \pm 0.01 \text{ mg N h}^{-1} \text{ Kg}^{-1}$ ). DP in the emergent macrophyte community was approximately 4 times higher in the upper layer than in the 9-18 cm layer. In the open water community, DP was 2.5 times higher in the 9-18 cm layer than in the 0-9 cm layer. In the forested

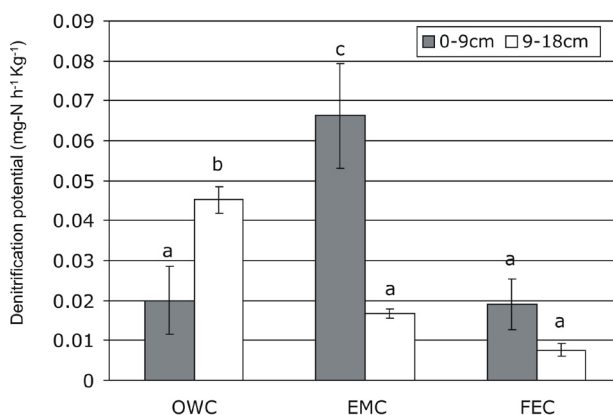


Figure 2. Denitrification potential of different vegetation community soils in created marshes. Values are means ( $n = 8$ ), bars represent standard error, and different letters indicate significant differences at levels of  $\alpha = 0.05$ . OWC = open water community; EMC = emergent macrophyte community; FEC = forested edge communities.

edge community, DP was low and not significantly ( $P > 0.05$ ) different between the two layers.

Organic matter (OM) content in the surface layer was significantly higher in emergent macrophyte community ( $84.90 \pm 5.60 \text{ g Kg}^{-1}$ ) than in open water community ( $62.70 \pm 2.90 \text{ g Kg}^{-1}$ ) and forested edge community ( $64.30 \pm 5.10 \text{ g Kg}^{-1}$ ) (Figure 3a). In the 9-18 cm layer OM was not significantly different ( $P > 0.05$ ) among the communities. Only in the emergent macrophyte community, was OM significantly higher ( $P < 0.05$ ) in the 0-9 cm layer than the deeper layer.

Cold water extractable organic matter (CWEOM) in the 0-9cm layer was 8 times higher in the emergent macrophyte community than in open water community and approximately 4 times higher than in the forested edge community (Figure 3b). On the other hand, CWEOM in the 9-18-cm layer was 2.5 times higher in open water community than emergent macrophyte community and 7 times higher than forested edge community. CWEOM in the forested edge community did not differ between the two depths. Hot water extractable organic matter (HWEOM) was significantly higher ( $P < 0.05$ ) in the emergent macrophyte and open water surface

Table 1. Denitrification potential and organic matter characteristics in soils covered with different emergent macrophyte species. Values are means  $\pm$  SE ( $n = 8$ ).

Parameter	Plant species			
	<i>Schoenoplectus tabernaemontani</i>		<i>Typha</i> spp.	
	0-9 cm	9-18 cm	0-9 cm	9-18 cm
DNP ( $\text{mg N h}^{-1} \text{ Kg}^{-1}$ )	$0.04 \pm 0.01^a$	$0.02 \pm 0.00^a$	$0.09 \pm 0.02^b$	$0.02 \pm 0.00^a$
OM ( $\text{g Kg}^{-1}$ )	$67.50 \pm 5.90^a$	$60.01 \pm 9.90^a$	$102.30 \pm 3.40^b$	$47.90 \pm 4.02^a$
CWEOM ( $\text{g Kg}^{-1}$ )	$0.81 \pm 0.20^a$	$0.14 \pm 0.08^b$	$1.56 \pm 0.14^c$	$0.26 \pm 0.05^b$
HWEOM ( $\text{g Kg}^{-1}$ )	$1.60 \pm 0.20^a$	$0.58 \pm 0.05^b$	$1.30 \pm 0.05^a$	$0.70 \pm 0.04^b$
AnMC ( $\text{mg C h}^{-1} \text{ Kg}^{-1}$ )	$1.09 \pm 0.12^a$	$0.49 \pm 0.04^b$	$1.75 \pm 0.08^c$	$0.28 \pm 0.17^b$

Means in the same row followed by the same letter are not significantly different ( $P < 0.05$ )

DP= Denitrification potential, OM=Organic matter, CWEOM=Cold water extractable organic matter, HWEOM= Hot water extractable organic matter and AnMC=Anaerobic mineralizable carbon

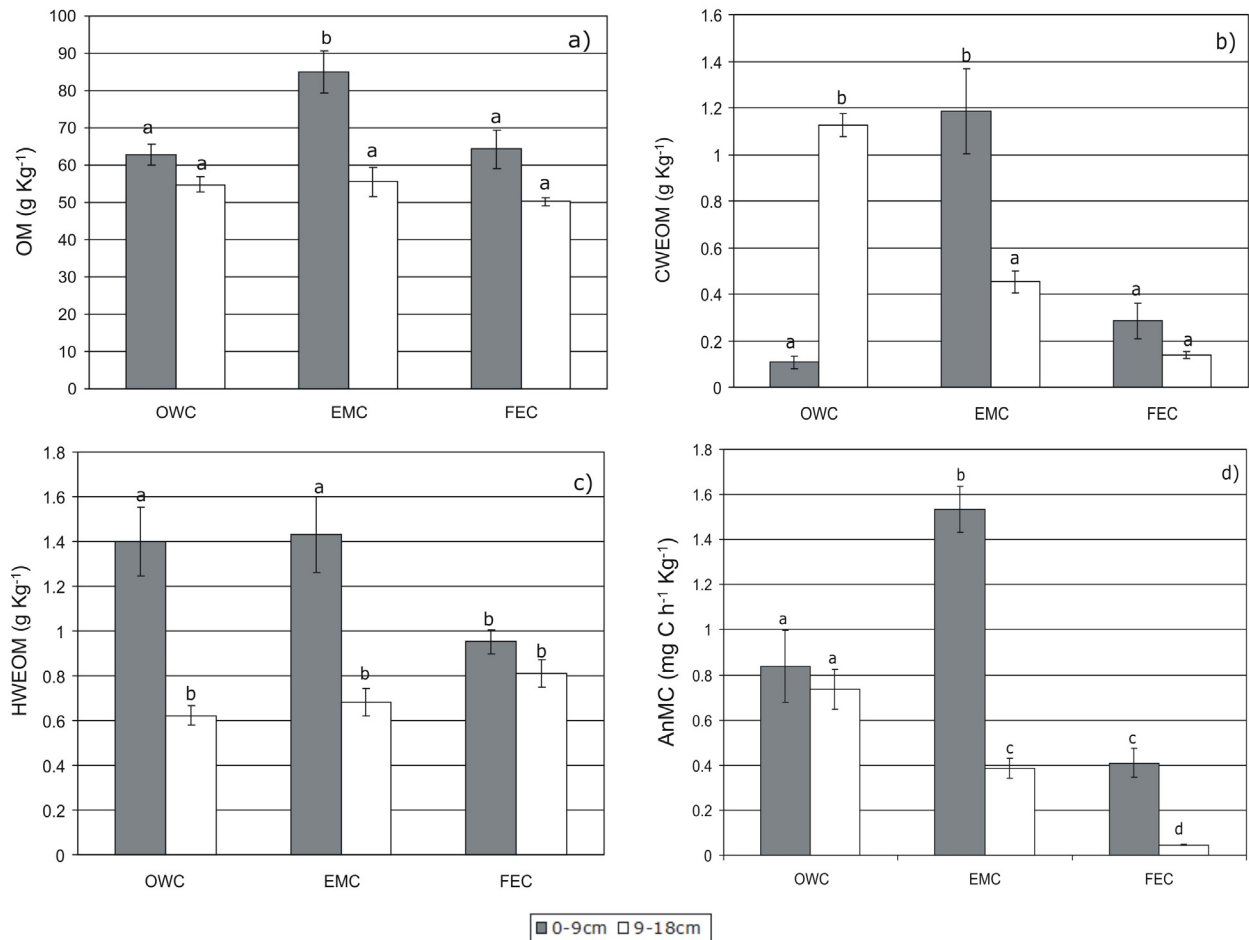


Figure 3. Organic matter characteristics in the different vegetation communities in created wetlands: a) organic matter (OM) content; b) cold water extractable organic matter (CWEOM) content; c) hot water extractable organic matter (HWEOM) content; and d) carbon mineralized under anaerobic incubation (AnMC). Values are means ( $n=8$ ), bars represent standard error, and different letters indicate significant differences at levels of  $\alpha = 0.05$ . OWC = open water community; EMC = emergent macrophyte community; FEC = forested edge communities.

soils than in the forested edge surface soils; in the deeper layer no differences on HWEOM were observed among the vegetation communities (Figure 3c). The highest anaerobic mineralizable carbon (AnMC) was observed in the 0-9 cm layer of emergent macrophytes followed by open water and forested edge (Figure 3d). AnMC was significantly higher ( $P < 0.05$ ) in the 0-9 cm than 9-18 cm layer in the emergent macrophytes and forested edge but AnMC in the open water was similar in both layers.

In the 0-9 cm layer DP, OM, CWEOM, and AnMC were significantly higher in the *Typha spp.* patches than in *Schoenoplectus tabernaemontani* patches (Table 1). However, in the 9-18 cm layer no significant differences were observed in these parameters. HWEOM was not significantly ( $P > 0.05$ ) different in any soil layer in the two patches.

Organic matter extracted by HCl/HF was very low in all samples, ranging from 2.2 to 3.1% of OM, and no significant differences were observed either among the vegetation communities or among the layers (Figure 4). Fulvic acids extracted at pH 7 ranged from 3.9 to 4.2% of OM, and

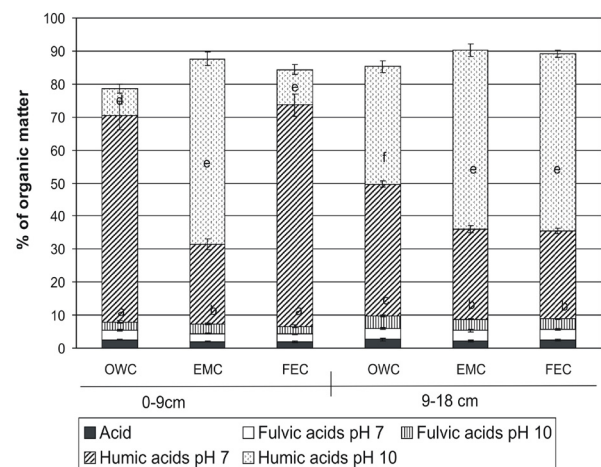


Figure 4. Humic substances content in soils covered with different vegetation communities in created marshes. Values are means ( $n = 8$ ), bars represent standard error, and different letters indicate significant differences at levels of  $\alpha = 0.05$ . OWC = open water community; EMC = emergent macrophyte community; FEC = forested edge communities.

fulvic acids extracted at pH 10 ranged from 2.1 to 3.1% of OM. No significant differences in fulvic acid content were found among the samples or among the layers. Humic acids were the most abundant forms of organic matter in all vegetation communities and differences in their solubility were observed among the vegetation communities. Humic acids extracted at pH 7 were most abundant in surface soils in open water and the forested edge (62 and 65% of OM) while humic acids extracted at pH 10 were most abundant in emergent macrophyte soils (53% of OM). In the 9-18 cm layer, humic acids extracted at pH 7 were also more abundant in open water soils (40% of OM) while in the emergent macrophyte and forested edge soils, humic acids extracted at pH 10 (54 and 53%, respectively) were most abundant.

### Comparison of planted and unplanted wetlands

Ten years after plants were introduced to one wetland basin (W1) but only allowed to colonize naturally in the other wetland basin (W2), no effects of plant introduction on DP, OM, CWEOM, HWEOM and AnMC were observed (Table 2). This pattern was observed in both soil layers.

### Denitrification potential and organic matter in soils before and after wetland creation

Mean DP were very low in both 0-9 cm layer ( $0.002 \pm 0.001 \text{ mg h}^{-1} \text{ Kkg}^{-1}$ ) and in the 9-18 cm layer ( $0.001 \pm 0.001 \text{ mg h}^{-1} \text{ Kkg}^{-1}$ ) and no significant differences were observed between the two layers in the 1993 soil samples (Figure 5). In 2004, 10 years after the wetlands were initially flooded and 11 years after soil sampling, mean DP increased significantly ( $P < 0.05$ )—25-fold in the 0-9 cm layer ( $0.053 \pm 0.03 \text{ mg h}^{-1} \text{ Kkg}^{-1}$ ) and 15-fold in the 9-18 cm layer ( $0.015 \pm 0.004 \text{ mg h}^{-1} \text{ Kkg}^{-1}$ ). Mean OM in the alluvial soils before the wetlands creation was not significantly different between the layers ( $40.11 \pm 1.70 \text{ g Kkg}^{-1}$  in the 0-9 cm layer and  $33.63 \pm 5.56 \text{ g Kkg}^{-1}$  in the 9-18 cm layer) (Figure 6a). In 2004, mean OM in the 0-9 cm horizon increased significantly ( $P < 0.05$ ), approximately doubling to  $90.80 \pm 19.22 \text{ g Kkg}^{-1}$ . In the 9-18 cm layer, mean OM increased by 38% from 1993 to 2004 ( $46.93 \pm 3.85 \text{ g Kkg}^{-1}$ ) but this increase was not significant ( $P > 0.05$ ). Mean CWEOM in the 0-9 cm layer

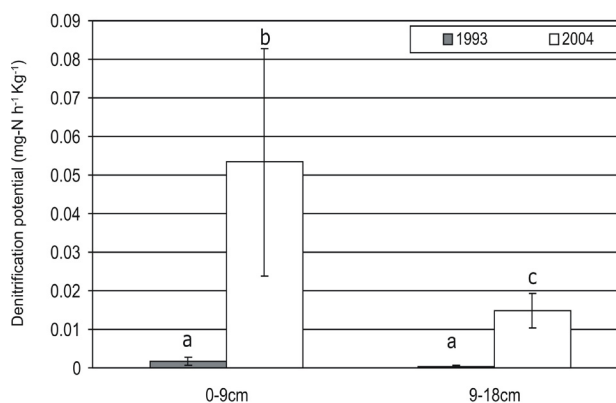


Figure 5. Comparison of denitrification potential (DP) in soils before wetland creation (1993) and 10 years after wetlands were flooded (2004). Values are means ( $n = 12$ ), bars represent standard error, and different letters indicate significant differences at levels of  $\alpha = 0.05$ .

increased significantly from  $0.35 \pm 0.02 \text{ g Kkg}^{-1}$  in 1993 to  $1.10 \pm 0.40 \text{ g Kkg}^{-1}$  in 2004 (Figure 6b). In the 9-18 cm layer, mean CWEOM increased from  $0.2 \pm 0.05 \text{ g Kkg}^{-1}$  in 1993 to  $0.5 \pm 0.10 \text{ g Kkg}^{-1}$  in 2004. Mean HWEOM in the 0-9 cm layer increased from  $0.4 \pm 0.03 \text{ g Kkg}^{-1}$  in 1993 to  $1.10 \pm 0.04 \text{ g Kkg}^{-1}$  in 2004 (Figure 7c). In the 9-18 cm layer, mean HWEOM also increased from  $0.24 \pm 0.02 \text{ g Kkg}^{-1}$  in 1993 to  $0.60 \pm 0.02 \text{ g Kkg}^{-1}$  in 2004. In 1993, mean AnMC was significantly higher in 9-18 horizons ( $0.76 \pm 0.10 \text{ mg h}^{-1} \text{ Kkg}^{-1}$ ) than 0-9 cm ( $0.42 \pm 0.0 \text{ mg h}^{-1} \text{ Kkg}^{-1}$ ) (Figure 7d). In 2004, mean AnMC increased to  $1.80 \pm 0.20 \text{ mg h}^{-1} \text{ Kkg}^{-1}$  in the 0-9 cm layer, but no increase was observed in the 9-18 cm layer.

Humic acids were also the main component of organic matter. Humic acids extracted at pH 7 were significantly higher in 1993 soils taken before the wetlands were created than in 2004 samples that have been flooded for 10 years (Figure 7).

### Relationships between denitrification potential and organic matter characteristics

In the 0-9 cm layer, we found a significant positive relationship between OM content and DNP (Pearson

Table 2. Effect of plant introduction on denitrification potential and organic matter characteristics in soils of created wetlands. Values are means  $\pm$  SE ( $n = 16$ ).

Parameter	W1 Planted		W2 Not planted	
	0-9 cm	9-18 cm	0-9 cm	9-18 cm
DNP ( $\text{mg N h}^{-1} \text{ Kkg}^{-1}$ )	$0.04 \pm 0.01$	$0.03 \pm 0.01$	$0.05 \pm 0.01$	$0.03 \pm 0.01$
OM ( $\text{g Kkg}^{-1}$ )	$70.60 \pm 3.50$	$52.20 \pm 1.90$	$79.10 \pm 0.90$	$52.70 \pm 0.36$
CWEOM ( $\text{g Kkg}^{-1}$ )	$0.50 \pm 0.11$	$0.60 \pm 0.14$	$0.80 \pm 0.30$	$0.50 \pm 0.10$
HWEOM ( $\text{g Kkg}^{-1}$ )	$1.10 \pm 0.10$	$0.77 \pm 0.05$	$1.50 \pm 0.01$	$0.63 \pm 0.03$
AnMC ( $\text{mg C h}^{-1} \text{ Kkg}^{-1}$ )	$1.00 \pm 0.14$	$0.40 \pm 0.10$	$1.10 \pm 0.18$	$0.40 \pm 0.10$

DP= Denitrification potential, OM=Organic matter, CWEOM=Cold water extractable organic matter, HWEOM= Hot water extractable organic matter and AnMC=Anaerobic mineralizable carbon

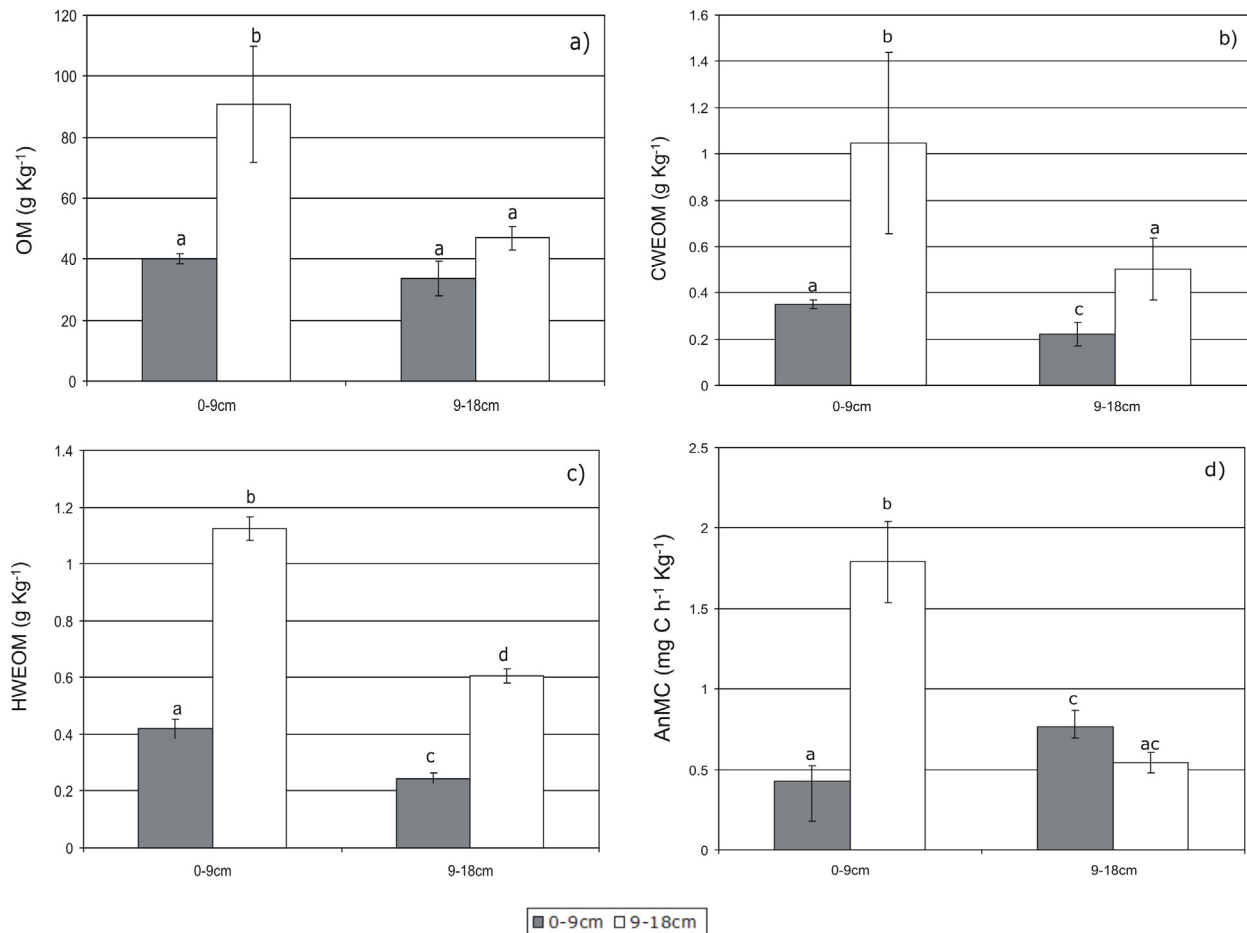


Figure 6. Comparison of soil organic matter characteristic before (1993) and 10 years after wetlands were flooded (2004). a) Soil organic matter (OM) content; b) cold water extractable organic matter (CWEOM) content; c) hot water extractable organic matter (HWEOM) content; and d) carbon mineralized under anaerobic incubation (AnMC). Values are means ( $n = 12$ ), bars represent standard error, and different letters indicate significant differences at levels of  $p = 0.05$ .

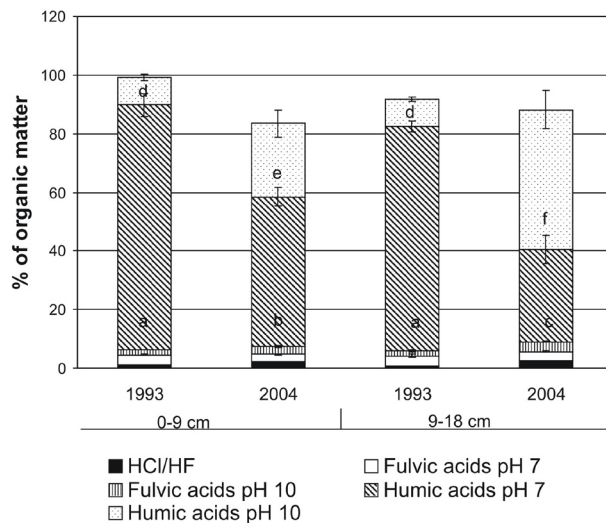


Figure 7. Comparison of humic substances content in soils before wetland creation (1993) and 10 years after wetland basins were flooded (2004). Values are means ( $n = 12$ ), bars represent standard error, and different letters indicate significant differences at levels of  $p = 0.05$ .

coefficient = 0.82), between CWEOM and DNP (Pearson coefficient = 0.81), and between AnMC and DNP (Pearson coefficient = 0.60) (Table 3). We did not observe significant relationships between HWEOM and DNP (Pearson coefficient = 0.33). In the 9-18 cm layer, a significant positive relationship was found only between CWEOM and DNP (Pearson coefficient = 0.91), and AnMC and DNP (Pearson coefficient = 0.84).

## Discussion

### Vegetation type, organic matter content and denitrification potential

Denitrification requires anoxic conditions, organic carbon as an electron supply, and nitrate as a terminal electron acceptor (Beauchamp et al., 1989). Organic matter accumulation in wetlands is the net result of primary production and decomposition. Decomposition of dead plant material is slowed significantly in wetlands due to anaerobic conditions; this results in the formation of extensive peat deposits (Debusk and Reddy, 1998; Collins and Kuehl, 2000).



In this study we observed that denitrification potential and quantity of organic matter in created wetlands soils varied among different vegetation type. Emergent macrophytes communities had higher organic matter content and denitrification potential than open water zones and forest edge zones. Our results agreed with a previous detailed spatial study of organic matter in these wetlands, which found greatest concentrations of organic matter along the emergent zone and lowest concentrations in the open water zone (Anderson et al., 2005).

The higher content of OM in the emergent macrophyte zones might be due to a combined effect of high productivity and different chemical composition of structural carbohydrates.

Detrital organic pool in wetlands consists of residual organic compounds of plant materials (Wetzel, 1985). Decomposition rates depend on structural composition of plants; emergent macrophytes contain higher percentage of structural carbohydrates such as cellulose and lignin than floating and submerged macrophytes in open water zones. Decomposition of lignin is slow because is a recalcitrant organic compound that is not easily hydrolyzed and metabolized.

In the emergent vegetation patches, organic matter was higher in the surface layers than in the subsurface layers. However, this pattern was not observed in the open water or edge zones. This could be due to the different structure of vegetation that was the source of organic matter in the different zones. In the emergent vegetation zone, soil organic matter sources are mainly both above ground and below-ground biomass decay. Hernandez et al. (2004) found that approximately 70% of below-ground biomass in these wetlands was in the 0-9 cm depth layer. Therefore, it is expected that this layer has higher organic matter inputs by both above-ground and below-ground biomass decomposition. On the other hand, in the open water zone, organic matter was not different in the two soil depths. In these zones, organic matter sources are both deposition of autothonous material (decomposed algal, floating and submerged macrophytes biomass) and allochthonous material (river sediments). Allochthonous material accumulation in these zones will dilute the samples since river sediments are low in organic matter relative to autothonous material.

The edge zones in these wetlands are dry most of the time with occasional flooding during flood pulsing; this condition favors colonization by the woody species. We believe that oxidation of organic matter in these areas is more rapid than in more frequently flooded marsh zones; therefore accumulation of organic in these edge patches was lower.

We found a significant linear relationship between denitrification potential and organic matter in the upper layer of these created riverine wetland soils similar to the linear relationship between denitrification and organic matter found in hardwood forest soils in the upper Rhine floodplain (Brettar and Hofle, 2002). In the deeper layer no linear

relationships between organic matter and denitrification potential were observed; we believe that this was due to the small variations in those parameters observed in different vegetation patches in this layer.

### Comparison of planted and unplanted wetlands

After 10 years of wetland development, no effect of plant introduction on denitrification potential and organic matter was observed. This may be due to the dynamic of macrophytes productivity over the time in these created wetlands. In 1997 both wetlands showed similar peak aboveground macrophyte productivity; then W2 (the naturally colonized wetland) showed higher macrophytes productivity than W1 (planted) for 4 straight years (1998-2002). In 2003 and 2004, W1 showed higher macrophyte productivity than W2. The cumulative organic matter produced by macrophytes in the last 7 years is now almost the same in both wetlands (Mitsch, 2005c). It is important to point out that the effect of plant introduction on denitrification potential and organic matter content and availability could have been different in earlier years of wetland development.

### Vegetation type, organic matter availability and denitrification potential

In experiments under laboratory conditions, the quality of organic carbon plays an important role as a limiting factor for denitrification in sediments of constructed wetlands and rivers. Addition of easily usable organic carbon such as soluble carbohydrates and acetate to the sediments caused higher denitrification rates than the addition of complex carbon molecules such as fulvic and humic acids (Pfenning and McMahon, 1996; Kozub and Liehr, 1999, Sirivedhin and Gray, 2006). Cold water extractable organic matter is a good indicator of physical mobility and availability of organic matter. Zsolnay and Steindl (1991) found that 85% of CWEOM in agricultural soils was mineralized and 15% was refractory. On the other hand, hot water extractable organic matter is more likely to be an indicator of the material that is potentially bioavailable (Zsolnay and Gorlitz, 1994). Hot water extractable organic matter from fertilized agricultural soil was composed of carbohydrates and N-containing compounds such as amino acids and amides (Leinweber et al., 1995). In this study we observed a significant linear relationship between CWEOM and denitrification potential in both layers. Similarly, significant correlations have been observed between DP and water soluble C, and DP and anaerobic mineralizable C in agricultura soils (Burdof and Bremner, 1975, Bijay-Singh et al., 1988). Also, in riparian buffer zones in Ontario Canada significant positive relationships between DP and water soluble C, and DP and anaerobic mineralizable were described by Hill and Cardaci (1995). Our results in created marsh soils agree with reports for agricultural soils (Buay-Singh et al., 1975) and riparian buffer zones (Hill and Cardaci, 1995). However, we did not find a linear relationship between HWEOM and



denitrification potential, which indicates that hot water extractable organic matter in these created wetlands, was not immediately available for anaerobic mineralization.

CWEOM and DP were higher in surface soil layers in *Typha* spp patches than in *Schoenoplectus tabernaemontani*. This probably is due to the type of parental material that these plants provide to soil organic matter. Plant material decomposition depends on structural composition of plants. *Typha* spp. contains approximately 10% of their dry mass as lignin and 40% as cellulose (Debusk and Reddy, 1998), while *Schoenoplectus* sp contains 35% of their dry mass as lignin (Hume et al., 2002). Lignin is more resistant to biological breakdown than cellulose. These differences in structural carbohydrates and also differences in plant productivity might be the reasons why *Typha* provides more available organic matter for denitrification than does *Schoenoplectus tabernaemontani*. Studies of decomposition of crop residues have revealed that cellulose eventually becomes a good carbon source for soil denitrifiers because there is an interaction between denitrifiers and anaerobic fermentative cellulose decomposers (Beauchamp et al., 1989).

CWEOM and DP were higher in 9-18 cm layer of the OWC; this indicates that organic matter in the deeper layer of this zone was more available than in the surface layer. In general, floating aquatic plants contain less lignin than emergent plants; therefore they are more easily degraded (Janssen and Walker, 1999). A decay coefficient ( $k$ ) of 3.7 yr<sup>-1</sup> has been reported for *Lemna gibba* (Szabo, et al., 2000), while for the emergent macrophyte *Phragmites australis*,  $k$  is in the range of 1.0 -2.5 yr<sup>-1</sup> (van der Valk et al., 1991). It has been suggested that due to relatively slow breakdown, emergent aquatic plants may be significant as a stable supply of carbon in variable environments. In contrast, litter from floating macrophytes may provide pulses of rapidly-utilizable carbon at different times a year. We believe that in the open water zones, allochthonous organic matter has been accumulated in the deeper layer providing more available organic matter than organic matter in the surface layer.

Denitrification fluxes (mg N m<sup>-2</sup> h<sup>-1</sup>) in created/constructed wetlands have been described in other studies (Poe et al., 2003, Srivedhin and Gray, 2005, Teiter and Mander, 2005); however, in our study we measured denitrification potential based in soil weight, thus we can not compared with other previous results in created wetlands. Comparing with denitrification potential in natural riparian wetlands, DP in these 10 years old created wetlands are 8 times lower than in riparian wetlands soils in Virginia Coastal Plain (Pavel et al., 1996) and 18 times lower that on riparian wetland (peat, mix forest and marsh) soils in Ontario Canada (Hill and Cradaci, 2004). This might be due to higher organic matter content in the natural riparian wetlands (100-360 g N kg<sup>-1</sup>) compared with an average of 85 g kg<sup>-1</sup> in the surface soils of these 10 years old created wetlands.

## Humic substance characteristics

Humic substances are a general category of naturally occurring heterogeneous organic substances that can be characterized as being yellow through black in color, high molecular weight, and refractory (Calace et al., 1999). Humification takes place under intense biological activity. In the first stage of the process, microorganisms decompose the original plant material into simpler compounds; later these compounds serve as components for the formation of humic acids, fulvic acids, and humin (Richarson and Vepraskas, 2000). In this study we found that humic substances were the major proportion of organic matter in created wetlands. In upland soils, 70-80% of soil organic matter is composed of humic substances (McGrath, 1987). Humic acids were the most abundant form of organic matter in all the vegetation patches in our created wetlands. Nagamitsu et al. (2002) found that rice paddy soils have a higher humic/fulvic ratio than upland soils because wetland conditions are more favorable to the formation of humic acids than fulvic acids. We did not find differences in humic acid content in the different vegetation patches but we did find differences in their solubility. Humic substance solubility gives us some information about their structure. For example, in Irish soil grasslands, humic acids extracted at pH 7 showed almost double the aromaticity than did humic acids isolated at pH 10 and 12 (McGrath, 1987); the same pattern was observed in podzols under oak trees and cleared forests (Simpson, et al. 1997). Aromaticity is related to the transformed state of organic matter; the more aromatic the more transformed. Although we did not investigate the composition of humic acids in this study, humic acids in edge and open water zones may be more transformed than in the emergent vegetation zone, based on their solubility. This was not expected since the hydrological conditions in the open water and the forested edge are different. A possible explanation may be the fact that in the open water zones there is accumulation of river sediments and these sediments probably originated from upland soil erosion. Flooded conditions seem to favor the production of less aromatic (transformed) humic acids. On the other hand, transitional upland soils (forested edges) and upland flood plain soils (before wetland creation) showed more transformed humic acids.

## Ecological implications

This study showed that wetlands creation enhanced the denitrification potential of floodplains, which is beneficial for mitigation of high nitrate concentrations in surface waters. We compare DP and OM in air-dried 1993 and 2004 samples. Organic matter content in the room temperature-stored 1993 samples was 25% lower than the mean organic matter content found by Nairn (1996) and described by Anderson et al. (2005). This may be the effect of the 11 years samples storage. Unfortunately we do not have a reference of DP measured in 1993; thus the effect of 11 years of sample storage on DP is unknown. Studies have shown that denitrification activity of air-dried soils with

nitrate amendments increased 7 times relative to the fresh samples after 1 week's storage but after 7 week's storage DP decreased and was only 1.5 times greater than fresh soil samples (Luo et al., 1996). It has been suggested that changes in denitrification activity on air dry samples might be due to changes in carbon availability and persistence of reduction enzymes (Buay-Singh et al., 1975; Luo et al., 1996). We found approximately 25 times more denitrification activity in the surface layer after 10 year of wetland development. Although this increase might partially be due to a storage effect on the 1993 soil samples, it appears that the potential for these floodplain wetlands to carry out denitrification is considerably higher 10 years after the wetlands were created compared to when they were constructed.

Our results suggest that vegetation type plays an important role in the quantity and quality of organic matter supply for denitrification in the 10-year-old created wetlands soils. Hydrology influences the establishment of different plant communities in wetlands (Mitsch and Gosselink, 2000). In these wetlands the hydrology is mostly controlled by river water pumped from the Olentangy River. Water enters to these wetlands at their north side, flows southwards through the wetland (figure 1), and finally returns to Olentangy, the residence time is between 1.5 to 4 days, depending of the inflow rate. Both wetlands have three deepwater (>50 cm depth) sections located in the inflow, middle and outflow positions of the basins, surrounded by much shallower sections (20-30 cm deep) dominated by emergent plants. Hernandez and Mitsch (in review) found that the highest denitrification rates in these wetlands occur in the shallow permanent flooded areas with emergent macrophyte vegetation near the inflow. Therefore, if wetlands with high denitrification rates are desired, their design should include shallow water areas to allow colonization of emergent macrophytes rather than large open water areas. Also, the self design of created wetlands for nitrate removal may be assisted by planting or seeding adequate high productive macrophytes that provide a stable supply of available organic matter for denitrification.

## Conclusions

Significant variations in denitrification potential and quantity, and availability of organic matter were found in zones with different vegetation communities in 10-year-old created wetlands. Emergent macrophytes zones showed high available organic matter and denitrification potential in the surface soil layers. Humic acids were the most abundant form of organic matter; in these wetlands soils, a significant variation in the solubility of humic acids was observed in the different vegetation communities. Denitrification potential in soils before the wetlands were created was one-twentieth that after 10 years; this increase seems to be related to the increase of quantity and availability of organic matter in the created wetlands.

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